

Appl. No.: 09/484,895

Amdt. Dated: August 27, 2003

Supplemental Reply to Office Action of: April 23, 2003

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

58. (currently amended) A vector comprising:

(a) a first promoter operably linked to an exon defined at its 3' end by an unpaired splice donor site, and

(b) a second promoter operably linked to a sequence encoding a selectable marker that lacks an operably-linked polyadenylation signal;

wherein said first and second promoters are present in said vector in the same orientation, and wherein both first and second promoters function in a eukaryotic cell and wherein said exon lacks a translational start codon or contains a translational start codon that is not operably linked to a translational stop codon.

59-60 (cancelled)

61. (previously amended) The vector of claim 58, wherein said vector is linear and wherein said second promoter is located 5' to said unpaired splice donor site.

62. (currently cancelled) The vector of claim 58, wherein said exon lacks a translation start codon.

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63. (currently cancelled) The vector of claim 58, wherein said exon comprises a translation start codon.

64. (currently amended) The vector of claim 58, wherein when said exon comprises a translation start codon, said exon also comprises ~~and~~ a signal secretion sequence operably linked to said translation start codon.

65. (previously amended) A vector comprising a first promoter and a second promoter, said first and second promoters being oriented in the same direction, wherein:

(a) said first promoter, but not said second promoter, is operably linked to an exon defined at its 3' end by an unpaired splice donor site; and

(b) said vector comprises no operably-linked polyadenylation signals downstream of either said first promoter or said second promoter and wherein both first and second promoters function in a eukaryotic cell.

66. (previously added) The vector of claim 65, wherein said vector is linear and wherein said second promoter is located 3' to said first promoter.

67. (previously amended) A vector comprising:

(a) a first promoter operably linked to a sequence encoding a first selectable marker and an unpaired splice donor site; and

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(b) a second promoter operably-linked to a sequence encoding a second selectable marker, wherein neither said first selectable marker sequence nor said second selectable marker sequence contains an operably-linked polyadenylation signal;

wherein said first and second promoters are present in said vector in the same orientation.

68. (previously amended) The vector of claim 67, wherein said first and second selectable marker sequences are positive selectable marker sequences.

69. (previously amended) The vector of claim 67, wherein said first selectable marker sequence is located upstream of said second selectable marker sequence.

70. (currently amended) A vector ~~construct~~ comprising:

(a) a first promoter operably linked to a sequence encoding a positive selectable marker;

(b) a second promoter operably linked to a sequence encoding a negative selectable marker; and

(c) an unpaired splice donor site,

wherein said splice donor site is 5' to said negative selectable marker and when said vector ~~construct~~ is integrated into the genome of a eukaryotic host cell and the vector-encoded splice donor is spliced to a splice acceptor in an endogenous gene in said genome, then said positive selectable marker sequence is expressed in active form and said negative selectable marker sequence is not expressed.

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71. (currently amended) The vector construct of claim 70, further comprising a third promoter operably linked to a second unpaired splice donor site.

72. (previously added) The vector of any one of claims 58, 65, 67, 70, or 71, said vector further comprising one or more transposition signals.

73. (previously amended) The vector of any one of claims 58, 65, 67, 70, or 71, said vector further comprising sequences encoding one or more amplifiable markers.

74. (previously added) The vector of any one of claims 58, 65, 67, 70, or 71, said vector further comprising one or more viral origins of replication.

75. (previously added) The vector of any one of claims 58, 65, 67, 70, or 71, said vector further comprising one or more viral replication factor genes.

76. (previously added) The vector of claim 73, wherein said amplifiable marker is selected from the group consisting of dihydrofolate reductase, adenosine deaminase, aspartate transcarbamylase, dihydro-orotase, and carbamyl phosphate synthase.

77. (previously added) The vector of claim 74, wherein said viral origin of replication is selected from the group consisting of Epstein Barr virus ori P and SV40 ori.

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78. (previously added) The vector of any one of claims 58, 65, 67, 70, or 71, said vector further comprising genomic DNA.

79. (previously amended) A eukaryotic host cell *in vitro* comprising the vector of any one of claims 58, 65, 67, 70, or 71.

80. (previously amended) A eukaryotic host cell *in vitro* comprising the vector of claim 72.

81. (previously amended) A eukaryotic host cell *in vitro* comprising the vector of claim 73.

82. (previously amended) A eukaryotic host cell *in vitro* comprising the vector of claim 74.

83. (previously amended) A eukaryotic host cell *in vitro* comprising the vector of claim 75.

84. (previously amended) A eukaryotic host cell *in vitro* comprising the vector of claim 78.

85. (previously amended) The eukaryotic host cell of claim 79, wherein said host cell is an isolated cell.

86. (previously amended) The eukaryotic host cell of any one of claims 80-85, wherein said host cell is an isolated cell.

87. (previously amended) A library of eukaryotic cells *in vitro* comprising the vector of any one of claims 58, 65, 67, 70, or 71.

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88. (previously amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 72.

89. (previously amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 73.

90. (previously amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 74.

91. (previously amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 75.

92. (previously amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 78.

93. (previously amended) A method for activation of an endogenous gene in a eukaryotic cell *in vitro* comprising:

(a) transfecting a eukaryotic cell *in vitro* with the vector of any one of claims 58, 65, 67, 70, or 71; and

(b) culturing said cell under conditions suitable for non-homologous integration of said vector into the genome of said cell, wherein said integration results in the activation of an endogenous gene in the genome of said cell.

94. (previously amended) A method for obtaining cDNA from an endogenous gene comprising:

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(a) transfecting a plurality of eukaryotic cells *in vitro* with the vector of any one of claims 58, 65, 67, 70, or 71;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of the cell;

(c) selecting for cells in which said vector has integrated into the genomes of said cells;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA; and

(f) isolating one or more cDNA molecules containing one or more nucleotide sequences from said vector.

95. (previously amended) The method of claim 94, wherein said isolation in step (f) is accomplished by hybridizing said cDNA in step (e) to said vector.

96. (previously amended) The method of claim 94, wherein said cDNA in step (e) or (f) is sequenced and the nucleotide sequence of said sequenced cDNA is compared to nucleotide sequence in said vector.

97. (previously amended) The vector of claim 67, wherein said unpaired splice donor site is positioned upstream of said first selectable marker sequence and when said vector is integrated into the genome of a eukaryotic host cell resulting in splicing from said unpaired splice donor site to a genome-encoded splice acceptor site, then said first selectable marker sequence is not expressed.

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98. (previously amended) A method for isolating eukaryotic cells *in vitro* in which a single exon gene has been activated, comprising:

(a) transfecting a plurality of eukaryotic cells *in vitro* with the vector of claim 97;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genomes of said cells; and

(c) selecting for cells in which said first and second selectable marker sequences are expressed in their active forms.

99. (previously amended) A method for isolating a single exon gene cDNA comprising:

(a) transfecting a plurality of eukaryotic cells *in vitro* with the vector of claim 97;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genomes of said cells;

(c) selecting for cells in which said first and second selectable marker sequences are expressed in their active forms;

(d) isolating RNA from the selected cells;

(e) producing cDNA from said isolated RNA; and

(f) isolating a single exon gene from said cDNA.

100. (previously amended) A method for isolating exon I of a gene comprising:

(a) transfecting one or more eukaryotic cells *in vitro* with the vector of any one of claims 58, 61, 65, or 67;

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(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells;

(c) selecting for cells in which said vector has transcriptionally activated an endogenous gene containing one or more exons;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA;

(f) recovering a cDNA molecule containing vector sequence and exon sequence from said endogenous gene; and

(g) using the exon sequence in the endogenous gene in (f) to obtain a cellular transcript or cDNA of a cellular transcript that contains the endogenous gene exon sequence and exon I of the endogenous gene.

101. (previously amended) A method for expressing a transcript containing exon I of a gene, said method comprising:

(a) transfecting one or more eukaryotic cells *in vitro* with the vector of any one of claims 58, 61, 65, or 67;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells; and

(c) culturing said cells under conditions suitable for expression of a transcript containing exon I from an endogenous gene.

102. (previously amended) A method for producing a gene product encoded by genomic DNA comprising:

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(a) isolating genomic DNA, containing at least one gene, from a eukaryotic cell;

(b) transfecting the vector of any one of claims 58, 61, 65, or 67 and said genomic DNA into a suitable eukaryotic host cell *in vitro* wherein the vector and genomic DNA are ligated or unligated; and

(c) culturing said host cell under conditions suitable to ligate said vector and genomic DNA and to result in transcription of one or more nucleic acid sequences in said vector.

103. (previously amended) A method for isolating a gene sequence comprising:

(a) isolating genomic DNA, containing at least one gene, from a eukaryotic cell;

(b) transfecting the vector of any one of claims 58, 61, 65, or 67 and said genomic DNA into a suitable eukaryotic host cell *in vitro* wherein the vector and genomic DNA are ligated or unligated;

(c) culturing said host cell under conditions suitable to ligate said vector and genomic DNA and to result in transcription of one or more nucleic acid sequences in said vector;

(d) isolating RNA produced by said transcription from said host cell;

(e) producing one or more cDNA molecules from said isolated RNA; and

(f) recovering one or more cDNA molecules containing vector sequences at the 5' ends of said cDNA molecules, thereby isolating said gene sequence.

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104. (previously amended) The method of claim 102, wherein said vector further comprises one or more transposition signals, and wherein said vector is ligated to said isolated genomic DNA by *in vitro* transposition.

105. (previously added) The method of claim 102, wherein said isolated genomic DNA is present in a cloning vector.

106. (previously amended) A method for producing a protein in a cell in which splicing can occur comprising:

- (a) isolating genomic DNA from one or more cells;
- (b) transfecting the vector of any one of claims 58, 61, 65, or 67 and said genomic DNA into a suitable host cell *in vitro* wherein the vector and genomic DNA are ligated or unligated; and
- (c) culturing said cell under conditions suitable to ligate said vector and genomic DNA and to result in protein expression from said genomic DNA.

107. (previously amended) The method of claim 106, wherein said host cell is selected from a cell containing said transfected vector and genomic DNA prior to, during, or following being cultured under conditions suitable to result in protein expression.

108. (previously added) The method of claim 105, wherein said cloning vector is selected from the group consisting of a BAC, a YAC, a PAC, a cosmid, a phage, and a plasmid.

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109. (previously added) The method of claim 102, further comprising isolating said protein.

110-112 (cancelled)

113. (previously amended) The vector construct of claim 70, wherein said positive selectable marker sequence is selected from the group consisting of a neomycin gene, a hypoxanthine phosphoribosyl transferase gene, a puromycin gene, a dihydroorotase gene, a glutamine synthetase gene, a histidine D gene, a carbamyl phosphate synthase gene, a dihydrofolate reductase gene, a multidrug resistance I gene, an aspartate transcarbamylase gene, a xanthine-guanine phosphoribosyl transferase gene, and an adenosine deaminase gene.

114. (previously amended) The vector construct of claim 70, wherein said negative selectable marker sequence is selected from the group consisting of a hypoxanthine phosphoribosyl transferase gene, a thymidine kinase gene, and a diphtheria toxin gene.

115. (previously amended) The vector of claim 70, wherein said negative selectable marker sequence is located upstream of said positive selectable marker.

116. (previously amended) The vector of claim 115, wherein said vector further comprises one or more selectable marker sequences.

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117. (previously amended) The vector of claim 67, wherein said unpaired splice donor site is positioned within said first selectable marker sequence and when said vector is integrated into the genome of a eukaryotic host cell resulting in splicing from said unpaired splice donor site to a genome-encoded splice acceptor site, then said first selectable marker sequence is expressed in inactive form.

118. (previously amended) A vector construct comprising:

- (a) a first promoter operably linked to a sequence encoding a positive selectable marker;
- (b) a second promoter operably linked to a sequence encoding a negative selectable marker; and
- (c) an unpaired splice donor site,

wherein said splice donor site is within said negative selectable marker and when said vector construct is integrated into the genome of a eukaryotic host cell and the vector-encoded splice donor is spliced to a splice acceptor in an endogenous gene in said genome, then said positive selectable marker sequence is expressed in active form and said negative selectable marker sequence is expressed in inactive form because of the splicing event.

119. (previously added) A method for isolating exon I of a gene comprising:

- (a) transfecting one or more eukaryotic cells *in vitro* with the vector of any one of claims 58, 61, 65, or 67;
- (b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells;

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- (c) selecting for cells in which said vector has transcriptionally activated an endogenous gene containing one or more exons;
- (d) isolating RNA from said selected cells;
- (e) producing cDNA from said isolated RNA;
- (f) recovering a cDNA molecule containing vector sequence and exon sequence from said endogenous gene; and
- (g) using the exon sequence in the endogenous gene to obtain genomic DNA containing exon I of the endogenous gene.

120. (currently added) A eukaryotic cell *in vitro* that contains a vector non-homologously integrated into its genome, said vector comprising a first transcriptional regulatory sequence operably linked to a selectable marker lacking an operably-linked poly-adenylation signal, said vector further comprising a second transcriptional regulatory sequence operably linked to an unpaired splice donor sequence, wherein transcription of an endogenous gene is activated by said integrated second transcriptional regulatory sequence and the activated transcript is translated into protein.

121. (currently added) A method for activating transcription and translation of an endogenous gene, said method comprising integrating a vector into the genome of a eukaryotic cell *in vitro*, said vector comprising a first transcriptional regulatory sequence operably linked to a selectable marker lacking an operably-linked poly-adenylation signal, said vector further comprising a second transcriptional regulatory sequence operably linked to an unpaired splice

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donor sequence, wherein transcription of an endogenous gene is activated by said integrated second transcriptional regulatory sequence and the activated transcript is translated into protein.